79-006 34

Prog. Wat. Tech. 1979, Suppl. 1, pp. 111-130.
Pergamon Press. Printed in Great Britain.

# TEMPERATURE DEPENDENCY OF MICROBIAL REACTIONS

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## ABSTRACT

The influence of temperature on the stoichiometry and rate of microbial processes is reviewed. Microbial rate processes are segregated into more fundamental processes such as mass transfer, biomass production, biomass decay and substrate uptake to provide a more systematic basis for analysis. The application of the techniques to determine temperature dependence of several unit processes is demonstrated with case studies based on published data.

## KEYWORDS

Biological treatment, temperature, mass transfer, microbial reactions

## INTRODUCTION TO MICROBIAL REACTIONS

Reaction processes have two features of importance to the engineer:

(1) Stoichiometry which indicates what changes will occur and to what extent.

(2) Rate which describes how fast the changes will occur.

The search for factors that influence process stoichiometry and rate must be accomplished in order to satisfactorily design and control technical scale processes and laboratory experiments. Temperature is major process variable.

### Characteristics of Microbial Reactions

The methods for determining the stoichiometric and kinetic parameters for a given conversion have been developed primarily for abiotic reactions. Biochemical conversions mediated by viable organisms differ from abiotic chemical conversions in several ways:

(1) Microbial reactions are irreversible. The stoichiometric endpoint generally refers to the exhaustion of one of the reactants.

(2) All are heterogeneous.

(3) Relatively low concentrations of reactants and products increase the potential for mass transfer limitations.

(4) The reaction, frequently represented by one stoichiometric equation, consists of many enzymatic reactions (in series and parallel) occurring in the metabolic region of the cell.

(5) The reactions are autocatalytic, i.e., biomass and related enzymes increase as the reaction proceeds.

(6) One of the reactants, biomass, has a structure which can influence the stoichiometry and kinetics of the reaction, i.e., there are different species of microorganisms each capable of performing different conversions in the same reaction environment.

## Microbial Stoichiometry

The microbial reaction can be expressed generally as follows:

$$\text{energy source} + \text{carbon source} + \text{electron acceptor} + \text{inorganic nutrients} + \text{growth factors} \xrightarrow{\text{(biomass)}} \text{more biomass} + \text{other synthesis products} + \text{respiration products} \quad (1)$$

Microbial reactions can therefore be conveniently classified according to the energy source, carbon source and electron acceptor (donor) utilized for growth and reproduction (Table 1). This paper is primarily concerned with aerobic chemosynthetic reactions, although the principles apply equally well to the other reactions.

TABLE 1 Classification of Microbial Reactions Based on Reactants

| ENERGY SOURCE | CARBON SOURCE | ELECTRON Acceptor/Donor |
|---|---|---|
| Photosynthetic-light energy | Autotroph-inorganic carbon | Aerobic-oxygen present |
| Chemosynthetic-chemical energy | Heterotroph-organic | Anaerobic-oxygen-absent |

So long as the reactants are present in excess, the reaction proceeds in an autocatalytic manner. However when the ratio of one of the reactants to biomass is low, biomass decay significantly influences the process. Aerobic biomass decay can be described as follows:

$$\text{biomass} + \text{oxygen} \longrightarrow \text{oxidized carbon} + \text{inorganic nutrients} \quad (2)$$

## Mass Transfer Considerations

Another useful method for classifying reactions is according to the number and types of phases[1] involved. A reaction is homogeneous if it takes place in one phase alone. A reaction is heterogeneous if it requires the presence of at least two phases to proceed at its characteristic rate. A phase implies uniform composition. Consequently, the choice of classification is sometimes difficult and depends on which description is more useful. Rigorously, all microbial reactions are heterogeneous since the biomass is a "solid" phase and the substrate being consumed is generally considered to be in solution.

In homogeneous and heterogeneous reactions, temperature and composition are important variables. However in heterogeneous systems, reactants must be transported from one phase to another during reaction. Consequently, mass transfer processes may limit overall process rate. Mass transfer is temperature-dependent but is also affected by mixing intensity and system geometry. In any case, if the conversion consists of a number of rate processes in series, the slowest step of the series exerts the greatest influence and controls the overall rate. Consequently, changes in temperature not only change the individual rates but can result in a change in the "rate-controlling step."

## Mixed Microbial Populations

In mixed microbial populations, or enrichment cultures, species predominance is influenced by composition and temperature of the environment. Chemosynthetic growth occurs at the expense of energy released by the flow of electrons from donors to acceptors mediated by the viable organisms. A portion of this energy is captured by the organism for conducting the work of synthesis and reproduction. The remaining energy escapes as heat. The extent to which microbial growth occurs (stoichiometry) is a function of the energy released by the electron transfer (a characteristic of the energy source) and the efficiency of energy utilization by the organism (a characteristic of the microbial species). Those organisms which can convert the energy most rapidly (kinetics) and can capture released energy most efficiently (stoichiometry) in a given environment will dominate since their growth rate will be highest. The temperature dependence of microbial reaction stoichiometry and rate varies with the microbial species. Therefore, temperature strongly influences the microbial species distribution, even in an aqueous environment of constant chemical composition, due to its effect on species competition.

[1] A phase is that part of a system which is physically and chemically uniform throughout.

Summary

The concentrations of reactants and products in a continuous flow microbial reactor at "steady state" are determined by the reaction stoichiometry of the microbial population and the relative rates of microbial growth and decay. Determining or anticipating the effect of temperature in design or process control is the subject of this paper. The effects of temperature on the various factors influencing the "steady state" condition are discussed and a concluding section on process applications presents examples in order to further clarify the approach.

## STOICHIOMETRY

Reaction stoichiometry is determined by the coefficients in the reaction equation. For example, consider the following hypothetical reaction:

$$2\ A + B \longrightarrow C + 0.5\ D \qquad (3)$$

The stoichiometric coefficients (negative for reactants and positive for products) are -2, -1, 1 and 0.5 for A, B, C and D, respectively. After examining the reaction stoichiometry, preferably at more than one temperature, a decision must be reached as to whether to consider a single reaction or a number of reactions. When more than one stoichiometric equation is used to represent the observed changes, then more than one rate expression is necessary to follow the changing composition due to the multiple reactions. This principle will be applied to the microbial processes. Stoichiometric coefficients for microbial processes are best determined in a closed (batch) system with explicitly defined initial and final states (Busch, 1971; Payne, 1970). The initial state is characterized by a relatively large amount of substrate and a small number of viable cells, i.e., it is substrate-saturated. (Fig. 1). The final state is achieved when the substrate is exhausted. The stoichiometry for microbial growth in such a system is exemplified by the following (Busch, 1971):

$$C_6H_{12}O_6 + 2.45\ O_2 + 0.71\ NH_3 \longrightarrow 0.71\ C_5H_7NO_2 + 2.45\ CO_2 + 4.58\ H_2O \qquad (4)$$

### Yield

The ratio of products formed to reactants consumed is generally expressed in terms of yield. For example, yield in equation (3) could be expressed as 0.25 moles D produced per mole of A reacted. Although yield can be based on any desired product and reactant, yield of biomass due to consumed substrate is of greatest interest in this paper. Data from the literature (Flegal and Schroeder, 1976; Monod, 1942, 1949; Ng, 1969; Senez, 1962; Sinclair and Stokes, 1963) indicate that biomass yield is constant over a relatively wide temperature range for many organisms and substrates. Yield is constant in the temperature range where growth rate behaves according to an Arrhenius function. Outside of this temperature range, yield has a different value. An illustration of this behavior is presented in Fig. 2.

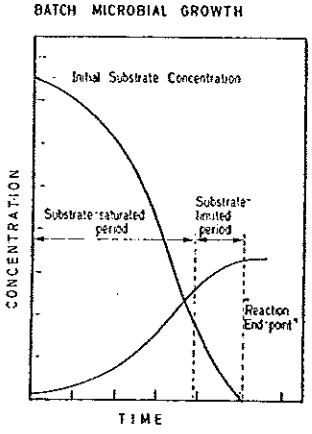



Fig. 1. Relationship between biomass and limiting nutrient concentration in a batch reactor assuming saturation kinetics.

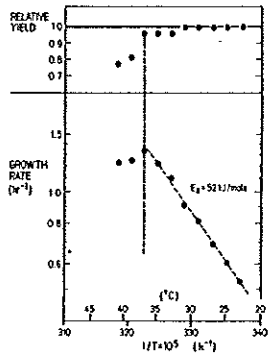



Fig. 2. Relationship between growth rate and yield for closed system growth as a function of temperature. Data from Monod (1942) for *B. coli* grown on glucose and mineral salts.

The good fit of growth rate to the Arrhenius model over a relatively wide temperature range seems to suggest the temperature dependence of a single reaction--the rate-limiting step. Outside of the characteristic temperature range, a change in rate-limiting step or a change in metabolic sequence may occur. A concurrent change in yield, especially in the latter case, would be expected. Monod (1949) has suggested that the reasonably good fit of microbial growth data to the saturation kinetics model is the fact that the overall rate is limited by one reaction--the rate-limiting step.

The temperature range in which yield remains constant may be greater than evidenced by much of the microbiological data. Sinclair and Stokes (1963) present data that indicate lower yields, frequently attributed to higher temperatures, can result from oxygen limitation. Higher oxygen solubilities and lower reaction rates at lower temperatures increases oxygen availability and, consequently, yield.

Flegal and Schroeder (1976) present data also indicating constant batch microbial reaction stoichiometry in enrichment cultures in the temperature range 10-35°C. Enrichment cultures might be expected to exhibit a wider temperature range in which stoichiometry or biomass yield remains constant because of the varying temperature sensitivities of the species initially present.

## Biomass Composition

In closed systems, biomass composition does not change as a function of temperature (Schaechter, Maaløe and Kjeldgaard, 1958). Biomass composition in continuous reactors, however, does change with temperature. Tempest and Hunter (1965) present an explanation based on intracellular activities, which rationalizes the discrepancies between composition changes in closed and open systems. Regardless of the intracellular mechanism, the behavior appears to be described well by presuming a biomass decay process whose rate is dependent on temperature:

$$\text{biomass} + O_2 \longrightarrow CO_2 + H_2O + \text{inorganic nutrients} \qquad (5)$$

Because of the rapid growth of the organisms in substrate-saturated, closed systems, biomass decay is insignificant. However in continuous reactors, biomass decay is a function of the imposed growth rate (i.e., dilution rate) and temperature (Tempest and Hunter, 1965) and suggests that multiple reactions (biomass production and decay) must be considered if the temperature effects are to be determined in a systematic manner.

$$\text{substrate} + O_2 + \text{nutrients} \longrightarrow \text{biomass} + \text{respiration product} \qquad (6)$$

$$\text{biomass} + O_2 \longrightarrow \text{respiration products} + \text{nutrients} \qquad (7)$$

Figure 3 diagrammatically illustrates the relationship between the two microbial reactions and the abiotic oxidation of the substrate.

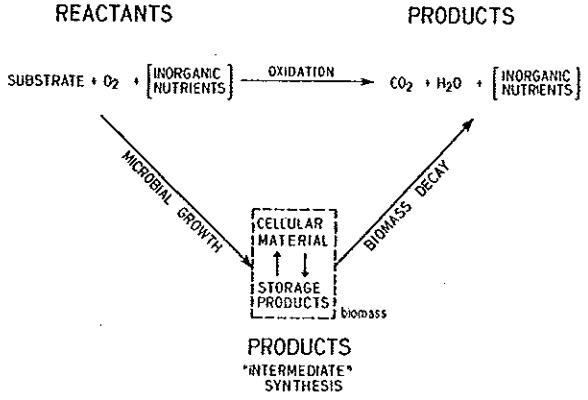



Fig. 3. The relationship between microbial and chemical reaction stoichiometries for oxidation of an organic nutrient.

## RATE CONSIDERATIONS IN MICROBIAL PROCESSES

The equations for the conservation of mass and chemical species in a continuous flow reactor equate the rate of accumulation to the net rate of input by flow and the net rate of input by various processes such as chemical reaction and diffusion (Fig. 4). The process rates are fundamental quantities in that they can be generalized and correlated simply with the factors that describe the process environment, e.g., temperature, composition and geometry. The rate of accumulation and the net rate of input by flow are rates of change which may be the result of several process rates. They cannot be correlated simply or generalized. The distinction between process rates and rates of change is vital. Unfortunately, it appears that much of the data in the literature on temperature effects reflects rates of change which are of little value in analyzing or designing systems where physical dimensions or reaction environment are not similar.

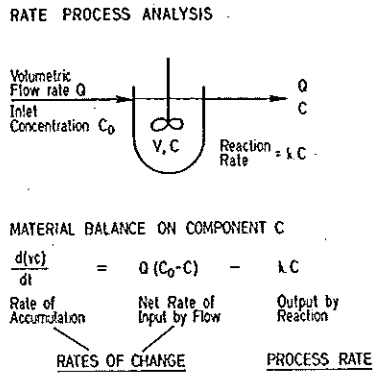



Fig. 4. Material balance for a continuous reactor in which a hypothetical first order reaction is occurring.

This section attempts to separate the various process rates that can affect biomass production and substrate removal so that the influence of temperature can be determined in a more systematic manner.

### Influence of Temperature on Rate

The effect of temperature on rate is generally described by the Arrhenius equation which implies that rate is an ever-increasing function of temperature:

$$k = A_f \exp[-E_a/RT] \tag{8}$$

where k = rate constant ($t^{-1}$)

$A_f$ = Arrhenius frequency factor ($t^{-1}$)

R = Universal gas constant (E $mole^{-1}T^{-1}$)
= 8.31 Joules/mole °K

T = temperature (T)

$E_a$ = activation energy (E $mole^{-1}$)

Rigorously, the Arrhenius equation only applies to single reactions or multiple reactions in which the first step is rate-limiting. Because of this and other reasons relating to its derivation, the concept of activation energy in microbial systems is not clear to the authors

at this time. Consequently, it is suggested that the stated activation energies in this discussion be considered as empirical constants which relate the process rate to temperature.

When the process rate is evaluated at two temperatures, the Arrhenius expressions can be combined to give

$$\ln (k_1/k_2) = E_a (T_1-T_2)/RT_1T_2 \tag{9}$$

When temperature differences are small,

$$\ln (k_1/k_2) = \theta (T_1-T_2) \tag{10}$$

and

$$\theta = E_a/RT_1T_2 \doteq 0.0015 E_a \tag{11}$$

where $E_a$ is in unit of kJ/mole.

$\theta$ and $10^{0.4340}$ are frequently used for relating process performance to temperature in the waste water treatment literature as is the $Q_{10}$ value. $Q_{10}$ indicates the fractional change in the rate (i.e., $k_1/k_2$) for a 10°C rise in temperature. Then

$$Q_{10} = \exp[E_a (10)/RT_1(T_1-10)] \tag{12}$$

or

$$Q_{10} \doteq \exp[E_a (0.014)] \tag{13}$$

Note that the $Q_{10}$ value for a process may vary according to the temperature interval used in the measurement.

## Mass Transfer vs. Reaction Rate Limitations

There are two complicating factors which must be considered in heterogeneous reaction systems beyond those considered in homogeneous reactions:

(1) Since more than one phase is present, the transport of components from one phase to another must be considered in the rate equation. Consequently, the rate expression will contain mass transfer terms as well as chemical reaction terms.

(2) Because of the different phases present, many different contacting patterns are possible. Each contacting pattern will generate a different mass transfer rate term so each rate equation will be different.

Microbial reactions are heterogeneous and mass transfer limitations can be significant as illustrated in Fig. 5 and described in many references (Atkinson, 1974; Characklis, 1978; Kornegay and Andrews, 1968; LaMotta, 1976a, 1976b; Mueller, Boyle and Lightfoot, 1966; Ngian, Lin and Martin, 1977; Powell, 1967). Such limitations are more evident with microbial aggre-

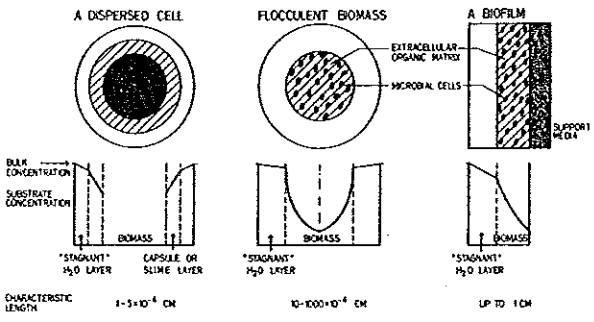



Fig. 5. Diagrammatic representation of mass transfer resistances in microbial systems with different contacting patterns based on published data and models.

gates such as flocs or films which are common to microbial waste water treatment processes. Most research or plant performance studies report rates in terms of the saturation kinetics model but fail to report the characteristic length of the biomass aggregates. Consequently, reaction rates and mass transfer rates cannot be separated and the effect of temperature on the process rate is somewhat difficult to analyze. Some information can be inferred from activation energy values since microbial growth rates generally have an activation energy of 50 kJ or more ($Q_{10} \doteq 2$) and diffusion processes exhibit a characteristic $E_a \doteq 18$ kJ/mole ($Q_{10} \doteq 1.3$).

An example calculation for a biofilm process may be instructive at this point. LaMotta (1976b) has indicated that the following expression describes substrate flux to a microbial film if zero order reaction kinetics within the biofilm is assumed:

$$N = (2D_e k_t)^{0.5} C_{sb}^{0.5} \tag{14}$$

where $N$ = substrate flux at the biofilm surface ($ML^{-2}t^{-1}$)

$D_e$ = effective diffusivity of substrate in the biofilm ($L^2t^{-1}$)

$k_t$ = "true" rate of substrate uptake by the organisms ($ML^{-3}t^{-1}$)

$C_{sb}$ = substrate concentration in the bulk fluid assuming no liquid phase mass transfer resistance ($ML^{-3}$)

For nitrification following secondary treatment (i.e., very little heterotrophic activity), a temperature increase from 10-20°C will result in $k_t$ increasing by a factor of 2.6 (Knowles and others, 1965) and $D_e$ will increase by 1.3. Based on equation (14), the substrate removal rate will increase by approximately 1.9 as a result of the temperature increase.

Enzyme Reaction (in vitro)

The rate of enzyme reactions as a function of substrate concentration is generally expressed as a saturation function which Michaelis and Menten (1913) developed from reaction mechanism considerations:

$$R = \frac{k_1 C}{k_2 + C} \tag{15}$$

where $R$ = reaction rate ($t^{-1}$)

$k_1$ = maximum reaction rate ($t^{-1}$)

$k_2$ = saturation constant ($ML^{-3}$)

$C$ = substrate concentration ($ML^{-3}$)

The effect of temperature on enzymatic reactions is illustrated in Fig. 6. The data indicate an increasing reaction rate from 1°C to approximately 53°C with $E_a \doteq 17.6$ kJ/mole. Above 53°C, the reaction rate decreases rapidly, presumably due to enzyme denaturation. Energies of activation for some other enzymes are listed in Table 2.

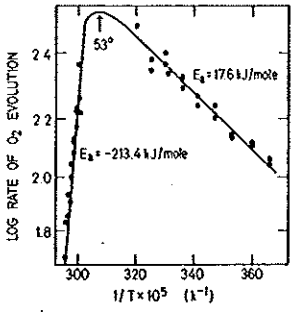



Fig. 6. Effect of temperature on the rate of decomposition of hydrogen peroxide catalyzed by crystalline catalase (Sizer, 1944).

TABLE 2 Activation Energies for Enzyme Activation and Thermal Inactivation (Denaturation) of Enzymes

| Enzyme | Energy of Activation (kJ/mole) | Process | Reference |
|---|---|---|---|
| urease | 28.4 | hydrolysis of urea | Laidler (1965) |
| myosin | 88.3 | hydrolysis of ATP | Laidler (1965) |
| catalase | 7.1 | decomposition of $H_2O_2$ | Laidler (1965) |
| catalase | 17.6 | decomposition of $H_2O_2$ | Sizer (1944) |
| catalase | -213.4 | denaturation | Sizer (1944) |
| pancreatic lipase | -192.5 | denaturation | Laidler and Bunting (1973) |
| ATPase | -292.9 | denaturation | Laidler and Bunting (1973) |

The data refer to the effect of temperature on $k_1$ since $C >> k_2$ in these experiments. Data on the effect of temperature on $k_2$ could not be found in the literature.

At constant temperature, a number of enzyme systems have been observed to denature irreversibly with time according to a first order rate expression:

$$\frac{dE}{dt} = - k'_d E \tag{16}$$

where E = concentration of active enzyme ($M/L^3$)

The decay constant, $k'_d$, depends on temperature, often in Arrhenius form (Bailey and Ollis, 1977).

## Microbial Reactions

The most widely used expressions for biomass production rate and substrate removal rate also utilize a saturation rate expression:

$$R_b = (\mu - k_d)C_b = \frac{\mu_m C_s C_b}{(K_s + C_s)} - k_d C_b \tag{17}$$

$$-R_s = \frac{\mu_m C_s C_b}{Y(K_s + C_s)} \tag{18}$$

where $R_b$ = rate of biomass production ($ML^{-3}t^{-1}$)

$R_s$ = rate of substrate removal ($ML^{-3}t^{-1}$)

$\mu$ = growth rate ($t^{-1}$)

$\mu_m$ = maximum growth rate ($t^{-1}$)

Y = maximum yield, i.e., Assuming no biomass decay

$K_s$ = saturation constant ($ML^{-3}$)

$k_d$ = biomass decay constant ($t^{-1}$)

$C_b$ = biomass concentration ($ML^{-3}$)

$C_s$ = substrate concentration ($ML^{-3}$)

Batch reactors. In batch reactors where the organisms are substrate-saturated, the microbial growth rate is characteristic of $\mu_m$ because $C_s >> K_s$ and $\mu_m >> k_d$. Experimental results from such systems (Flegal and Schroeder, 1976; Ingraham, 1958; Monod, 1942; Senez, 1962) indicate that $\mu_m$ (and hence, $\mu$) increases with temperature as described by an Arrhenius expression as illustrated in Fig. 2. Tables 3 and 4 list activation energies for $\mu_m$ and also thermal inactivation as reported in the literature. Changes in $K_s$ with temperature for batch reactors are indicated in Fig. 8.

TABLE 3 Activation Energies and Temperature Range (°C) for Maximum Microbial Growth Rate ($\mu_m$) Measured in Closed (Batch) Systems

| Activation Energy (kJ/mole) | Organism | Substrate | Reference |
|---|---|---|---|
| 52.3(23-33) | B. coli | glucose, mannitol sorbitol, maltose | Monod (1942) |
| 47.1(10-37) | enrichment | glutamic acid | Flegal and Schroeder (1976) |
| 69.4(20-30) | E. coli | glucose | Ng (1969) |
| 30.2(24.8-37) | D. desulfuricans | $N_2$ | Senez (1962) |
| 29.1(24.8-37) | D. desulfuricans | $NH_4^+$ | Senez (1962) |
| 42.6(23-37) | A. aerogenes | glucose | Senez (1962) |
| 63.2(18-39.4) | E. coli | glucose | Johnson and Lewin (1946) |
| 118.4(10-30) | P. aeruginosa | glucose | Brown (1957) |
| 71.1(0-20) | a psychrophile | glucose | Brown (1957) |
| 41.8(20-30) | a psychrophile | glucose | Brown (1957) |
| 145.6(0-15) | Pseudomonas | lactose | Green and Jezeski (1954) |
| 85.8(0-30) | Pseudomonas | lactose | Green and Jezeski (1954) |
| 45.6(8-20)<br>21.3(20-28) | G. fujikuroi | glucose | Borrow and others (1964) |
| 76.0(8.3-30.8) | Nitrosomonas | $NH_4^+$ | Knowles and others (1965) |
| 44.0(8.3-30.8) | Nitrobacter | $NO_2^-$ | Knowles and others (1965) |
| 77.9(6-14) | Nitrosomonas | $NH_4^+$ | Gujer (1977) |
| 68.9(6-14) | Nitrosomonas | $NH_4^+$ | Gujer (1977) |

TABLE 4 Activation Energies and Temperature Range (°C) for Thermal Inactivation of Microorganisms Measured in Closed (Batch) Systems

| Activation Energy (kJ/mole) | Organism | Substrate | Reference |
|---|---|---|---|
| -252 (37-42) | A. aerogenes | glucose | Senez (1962) |
| -167.5(37-43) | D. desulfuricans | $N_2$ | Senez (1962) |
| -173.9(37-42.2) | D. desulfuricans | $NH_4^+$ | Senez (1962) |
| -225.9*(40-50) | H. polymorpha | methanol | Cooney and Makiguchi (1976) |

* in a continuous reactor

Continuous reactors. In continuous reactors, growth rate is generally less than maximum and the concentration of the limiting nutrient is quite low. Consequently, the equations describing biomass production (and effluent nutrient concentration) contain three constants which depend on temperature (Fig. 7).

The effect of temperature on $\mu_m$, as measured in continuous reactors, is similar to that observed in batch reactors. Activation energies for $\mu_m$ range from 35-83 kJ/mole with a typical value of approximately 60 kJ/mole.

In continuous reactors, especially at lower dilution rates, growth rate is considerably less than maximum and biomass decay becomes significant. Biomass decay is a general term to account for processes such as endogenous metabolism, predation, maintenance requirements and cell lysis which tend to reduce biomass. The biomass decay coefficient, $k_d$, varies with temperature as indicated in Table 5, approximately doubling with a 10°C increase.

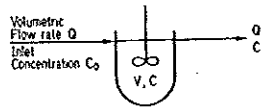
Biomass decay can obviously contribute to reduced levels of effluent biomass concentration from continuous reactors. Observed yield, $Y_o$, has been related to the biomass decay coefficient and solids retention time in the following manner (Sherrard and Schroeder, 1973):

$$Y_o = Y/(1 + k_d\theta_c) \tag{19}$$

where $\theta_c$ = solids retention time (t)

$Y_o$ = observed yield

Consequently, observed yield decreases with increasing temperature and increasing solids retention time.




MATERIAL BALANCE ON BIOMASS $C_b$

$$V\frac{dC_b}{dt} = Q(C_{b0}^{0} - C_b) + C_b V\left[\frac{\mu_m C_s}{K_s + C_s} - k_d\right]$$

$$\frac{Q}{V} = \mu_a = \frac{\mu_m C_s}{K_s + C_s} - k_d$$

$$C_s = \frac{K_s}{\left[\mu_m/(\mu_a + k_d)\right] - 1}$$

Fig. 7. Steady state material balance for biomass in a continuous reactor assuming saturation kinetics and first order (with biomass concentration) biomass decay.

The temperature effect on the "true" saturation constant, $K_s$, is not known. $K_s$ has frequently been related to mass transfer rates and so data from several investigations are presented in Arrhenius form in Fig. 8. The fact that the activation energies are positive for batch data and negative for continuous reactor data may indicate some inadequacy of the saturation kinetics form for description of both reaction systems.

## Substrate Removal Rate

From equation (17), it is obvious that substrate removal rate is a continuous function of $C_s$ and $C_b$. At high $C_s$, the removal rate is independent of $C_s$ and the reaction can be considered substrate-saturated. Under these conditions, the process is rate-limited by the microbial growth. This is the case for batch growth processes described in Tables 3 and 4. The effect of temperature on substrate removal rate in such cases is similar to that of maximum microbial growth rate, i.e., $E_a$ = 60 kJ/mole.

When removal rate is independent of $C_b$ (low $C_s$) the process is substrate-limited. This is the case for continuous reactors except for those operated at very high growth rates (near "washout"). Under substrate-limited conditions, substrate removal rate is not so sensitive to

TABLE 5 Activation Energies for Maximum Microbial Growth and Biomass Decay Measured in Continuous Reactors

| Activation Energies (kJ/mole) $\mu_m$ | $k_d$ | Organism | Substrate | Reference |
|---|---|---|---|---|
| 35.1 | 37.7 | A. aerogenes | glucose | Topiwala and Sinclair (1971) |
| 55.6 | - | E. coli | $NH_4^+$ | Ryu and Mateles (1968) |
| 69.5 | 76.1 | P. fluorescens | glucose | Mennet and Nakayama (1971) |
| - | 35.3 | P. fluorescens | glucose | Palumbo and Witter (1969) |
| 52.3 | 52.3 | enrichment | glucose | Muck and Grady (1974) |
| - | 55.0 | activated sludge | endogeneous | Benedek and Frakas (1971) |
| 70.3* | - | activated sludge | $NH_4^+$ (nitrification) | Downing and others (1964) |
| 57.9** | - | activated sludge | $NH_4^+$ (nitrification) | Downing and others (1964) |
| 83.2*** | - | activated sludge | $NH_4^+$ (nitrification) | Downing and others (1964) |
| 75.8 | - | activated sludge | $NH_4^+$ (nitrification) | Gujer (1977) |

* fill-and-draw system containing 50 mg/l biomass
** fill-and-draw system containing 500 mg/l biomass
*** continuous reactor containing 2000-6500 mg/l biomass

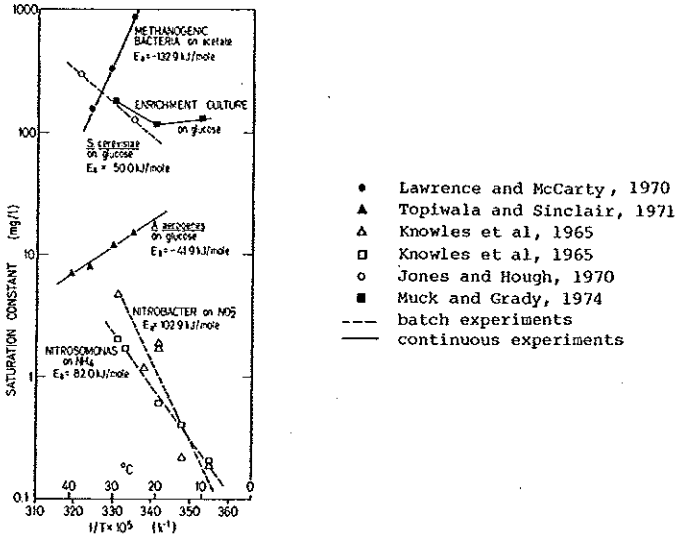



Fig. 8. Influence of temperature on the apparent saturation constant.

temperature. In further discussing this effect, it is useful to categorize the substrates as soluble or insoluble.

Soluble substrates. Nitrification provides a convenient example for the effect of temperature on substrate removal. The data of Knowles and others (1965), obtained from batch reactors, has been used in Fig. 9 to indicate the effect of temperature on residual substrate ($NH_4^+$) concentration in steady state continuous reactors at various growth rates. The results clearly indicate that residual substrate is independent of temperature at low growth rates and becomes increasingly more dependent on temperature as growth rate increases. In general, residual substrate will be higher at lower temperatures. Similar behavior is evident in removal of soluble organic substrates (Fig. 10). The data in Figs. 9 and 10 reflect rates of change as well as process rates as defined in Fig. 4.

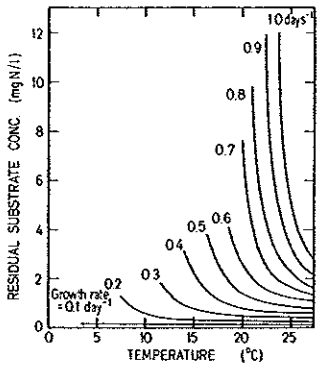



Fig. 9. Residual substrate concentration as a function of temperature and growth rate for *Nitrosomonas* with $NH_4^+$ as the substrate based on the batch reactor data of Knowles and others (1965).

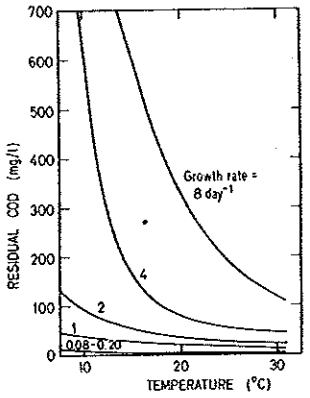



Fig. 10. Residual substrate concentration as a function of temperature and growth rate for an enrichment culture with glucose as the substrate based on data of Muck and Grady (1974) and Friedman and Schroeder (1971).

Insoluble substrates. The removal of colloidal and emulsified substrates by microbial activity appears to be mediated by an extracellular microbial product. For starch degradation, a hydrolytic enzyme is necessary (Banerji and others, 1968) while for hydrocarbon breakdown, some hydrophobic material is required (Mallee and Blanch, 1977).

Experiments with starch (Banerji and others, 1968; Maier, 1968) indicate that the hydrolytic enzymes are located in the microbial cell wall and that intimate contact of cell wall and starch was necessary for degradation. Since rapid adsorption of starch to the cells only occurred in acclimated cultures, adsorption was presumed to be related to inducible enzyme production. Banerji and others (1968) and Berg and Phillips (1968) suggest that the kinetics and stoichiometry of insoluble substrate removal are similar to those for soluble substrates except for the initial adsorption step. The degradation of starch resulting in synthesis and respiration occurs in two discernible steps:

(1) hydrolysis of the starch into smaller molecules
(2) metabolism of the smaller molecules.

The two rates appear to have different activation energies based on studies with activated sludge (Fig. 11). The discrepancy, however, may be due to transport limitation or even analytical techniques.

Hydrocarbon degradation is mediated by hydrophobic material produced in the cell wall (Mallee and Blanch, 1977). The hydrophobic material may even allow the transport of small hydrocarbon droplets through the cell wall. Only organisms that adhere to hydrocarbon droplets are able to utilize it as a substrate (Miura and others, 1977). In fact, hydrocarbon removal is higher for organisms which attach more tightly to the hydrocarbon droplet.

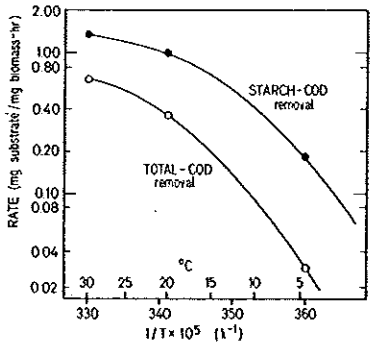



Fig. 11. Effect of temperature on starch removal rate (in terms of COD) and total COD for an enrichment culture growing on potato starch. Total COD refers to starch COD plus products resulting from hydrolysis of starch (Banerji and others, 1968).

## POPULATION DYNAMICS

Competition between microorganisms may result in the dominance of one species because of their short generation time and high population densities. In fact, the coexistence of several species at steady state in continuous culture is observed much more readily when more than one substrate is available. The diversity of nutrient sources in activated sludge, for example, must be a key factor in supporting a heterogeneous population of organisms. Yoon and others (1977) have demonstrated these principles in laboratory experiments with a two-substrate/two-organism system.

In an activated sludge plant, temperature has a significant effect on the population dynamics in the reactor. The resultant effect on plant performance may not necessarily be related to temperature by an Arrhenius function since temperature causes population shifts by altering growth rates of the individual species in different ways and changes the competitive situation between the species. Goldman and Ryther (1976) have effectively illustrated the effect of temperature on the population dynamics of algae in enriched continuous cultures in the laboratory. Five species were cultured axenically and in combination at temperatures varying from 10-30°C. Figure 12 indicates the change in steady state biomass carbon for the various species grown axenically. The effect of temperature on competitive advantage was tested in chemostat experiments described in Fig. 13. Axenic cultures of *S. costatum* were grown in a chemostat at 10 and 15°C. In each case, *S. costatum* was contaminated with *P. tricornutum*. At 10°C, *S. costatum* maintained its dominant position. However, at 15°C, *P. tricornutum* gained the dominant position after 6-8 days. The results presented in Fig. 13 may have been predicted from the axenic culture results in Fig. 12.

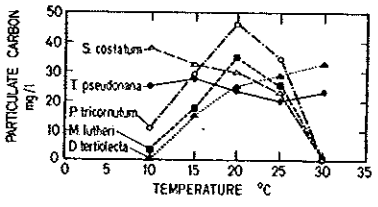



Fig. 12. Steady-state levels of particulate carbon as a function of temperature for 5 species grown in laboratory continuous cultures (Goldman and Ryther, 1976).

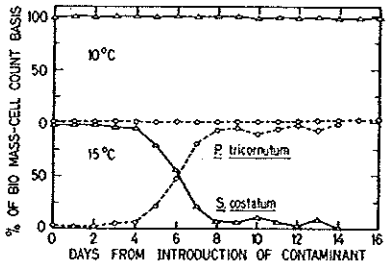



Fig. 13. Competition between *S. costatum* and *P. tricornutum* at 10 and 15°C. In both studies, monocultures of *S. costatum* were contaminated with *P. tricornutum* (Goldman and Ryther, 1976).

## PROCESS APPLICATIONS

This section will attempt to demonstrate how knowledge of the temperature dependency of process rates may be applied to the description of continuous processes. The four case studies are chosen to present a wide variety of temperature dependencies.

### Conversion Rates in the Activated Sludge Process

Gujer and Jenkins (1975) have shown that the following equation is applicable to any activated sludge process at steady state:

$$q = \mu_n \cdot i + r_h \tag{20}$$

where $q$ = COD removal rate (kg COD/kg VSS-day)

$\mu_n$ = activated sludge net growth rate ($days^{-1}$) (inverse of solids retention time)

$i$ = conversion factor (kg COD/kg VSS)

$r_h$ = total respiration rate of heterotrophic organisms (kg $O_2$/kg VSS-day)

This equation is a mass balance for oxygen equivalents in organic material removed and activated sludge formed.

Table 6 demonstrates how the parameters in equation (20) can be estimated for different process temperatures. In step 1, the initial values of the four parameters at 21°C are based on data of Gujer and Jenkins (1975). Wuhrmann (1964) observed the respiration rate of washed activated sludge - i.e., after removal of soluble substrates - as a function of solids retention time. The respiration rate of washed activated sludge, $r_w$, is due to endogenous respiration, degradation of flocculated colloidal sewage particles, biomass decay, etc. Wuhrmann reports a $Q_{10} = 2$ for this fraction of the total respiration rate and, in step 2, $r_w$ is adjusted to the desired process temperature. The difference between the total heterotrophic respiration rate, $r_h$, and $r_w$ is due to the assimilation of soluble organic substrates (characterized by $r_s$). As indicated earlier, $r_s$ is nearly independent of temperature at low growth rates so $r_s$ is presumed equal at both temperatures (step 3). In step 4, the total respiration rate, $r_h$, is predicted at the desired process temperature. Based on equation (20), the net growth rate, $\mu_n$, can be predicted (step 5). Observed and predicted growth rates at the alternative process temperature are within 5% (step 6) even though $\mu_n$ increased by 40% when the process temperature decreased from 21 to 11°C.

This example illustrates the usefulness of fundamental data on temperature effects in microbial reactors as described in the preceding sections. "True" stoichiometry is relatively unaffected by temperature. The individual process rates (biomass decay and substrate removal rate) and their temperature dependence have been segregated and recombined to yield information regarding overall rate of change.

### Removal Efficiency of the Activated Sludge Process

Substrate removal rate in continuous reactors is essentially independent of temperature at low growth rates as illustrated by the data on $NH_4^+$-N and glucose removal (Figs. 9 and 10). The activated sludge process is usually operated at very low growth rates and, consequently, substrate removal rate should exhibit little sensitivity to temperature. However, the activated sludge process is seldom controlled or evaluated based on the degradation of a specific compound. Collective parameters such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) or Total Organic Carbon (TOC) are used instead and sometimes introduce complications in data interpretation.

Wuhrmann (1964) has observed the removal efficiency of the activated sludge process (domestic wastes) under summer (>13°C) and winter (<11°C) conditions in terms of TOC and organic nitrogen removal (Fig. 14). Organic nitrogen removal is independent of temperature at low loadings as observed by Gujer and Jenkins (1975) and expected from the trends reported in Figs. 9 and 10. However, TOC removal appears sensitive to temperature even at low loadings - an inconsistent observation with regard to the previous discussions.

A TOC measurement is a non-specific analysis which encompasses organic compounds of varying degradability. The bio-degradation rate of some of these compounds may be highly temperature dependent. In addition, the TOC and TON measurement includes microbial products which are frequently produced to a greater extent at lower temperatures. In addition, some carbon

TABLE 6 Procedure to Predict Oxygen Requirement and Excess Sludge Production in the Activated Sludge Process (Data from Gujer and Jenkins, 1975).

| STEP | Quantity | Units | Value at 21°C | Value at 11°C |
|---|---|---|---|---|
| 1 | q | kg COD/kg VSS · day | 1.00 | 1.00 |
| | $\mu_n = 0.38\ q - 0.07$ | $day^{-1}$ | 0.31 | |
| | i | kg COD/kg VSS | 1.44 | 1.44 |
| | $r_h = q - \mu\ i$ | kg $O_2$/kg VSS-day | 0.55 | |
| 2 | $r_{25^o} = 12.5$ | g $O_2$/kg TS-hr | | |
| | Respiration rate of washed activated sludge at 25°C and $\mu = 0.31\ day^{-1}$ converted to process temperature using $Q_{10} = 2.0$ (Wuhrmann (55) | | | |
| | $r_w$ | kg $O_2$/kg VSS-day | 0.34 | 0.17 |
| 3 | Respiration rate due to assimilation of soluble organic compounds is independent of temperature | | | |
| | $r_s = r_h - r_w$ | kg $O_2$/kg VSS-day | 0.21 | 0.21 |
| 4 | $r_h = r_w + r_s$ | kg $O_2$/kg VSS-day | | 0.38 |
| 5 | Predicted growth rate at 11°C | | | |
| | $\mu_n = (q - r_h)/i$ | $day^{-1}$ | | 0.43 |
| 6 | Observed growth rate at 11°C | | | |
| | $\mu_n = 0.48\ q - 0.07$ | $day^{-1}$ | | 0.41 |

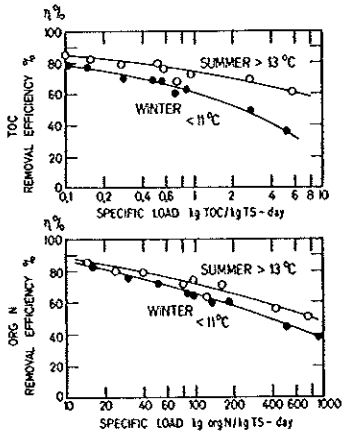



Fig. 14. TOC and org.N removal efficiency of an activated sludge process under summer and winter conditions (Data from Wuhrmann, 1964)

compounds may be volatile and their removal will be greater at higher temperature. If a specific analytical technique is used, the prediction of temperature effects on substrate removal rate will be more refined.

## Nitrification in the Activated Sludge Process

Lawrence and McCarty (1970) introduced the concept of a safety factor (SF) in the application of microbial kinetics to waste water treatment design. The SF relates the maximum growth rate of a population, $\mu_m$, to the imposed growth rate of the process, $\mu$:

$$SF = \mu_m/\mu$$

The SF indicates the potential of a process to accomodate peak loads and also indicates the margin of safety against "washout" of the microbial population.

Gujer (1977) has reported that the maximum growth rate of *Nitrosomonas* spp. in activated sludge varies with temperature in the same way as the maximum nitrification activity (Fig. 15 and 16). Fig. 17 relates nitrification efficiency of the activated sludge process to the SF for nitrifying organisms. Although $\mu_m$ for *Nitrosomonas* varies by a factor of 2.4 between 6 and 14°C (Fig. 16), the use of the dimensionless SF normalizes the temperature effect. The concept of relative growth rate, analogous to the SF, is useful when comparing organisms grown under different conditions and is discussed in more detail elsewhere (Evans and others, 1976).

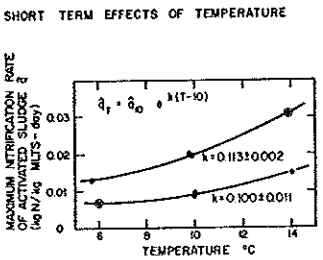



Fig. 15. Variation of nitrifier activity after a step change of the temperature. Double rings indicate temperature at which the activated sludge was grown. (Gujer, 1977).

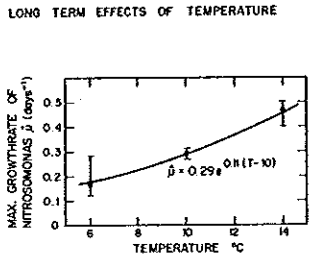



Fig. 16. Variation of *Nitrosomonas* max. growth rate with temperature. The organisms grew in laboratory scale activated sludge processes with a nitrification efficiency of approx. 50% for more than 10 weeks (Gujer, 1977).

The data in Fig. 17 are applicable to "steady state" only. The influence of diurnal temperature variation on nitrification in activated sludge has been considered with the aid of dynamic simulation techniques (Gujer, 1977).

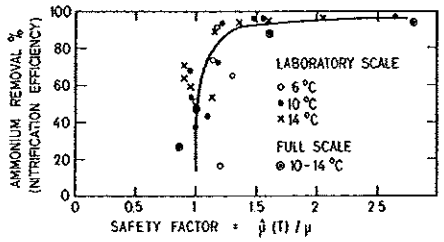



Fig. 17. Nitrification efficiency of an activated sludge process as a function of the dimensionless safety factor. Each data point is the average of at least one week of operation (Gujer, 1978).

The autocatalytic nature of microbial reactions results in complete nitrification at high SF since the density of nitrifying organisms in the activated sludge increases until the residual $NH_4^+$-N becomes quite small. At high SF, i.e., low growth rates, the nitrification efficiency becomes independent of temperature as indicated in Fig. 9.

## Nitrification in a Trickling Filter

Trickling filters have successfully been used to nitrify secondary effluents. The detailed modelling of the behaviour of a nitrifying biofilm is complex but a few simple calculations can illustrate how empirical nitrification data from trickling filters can be interpreted (Fig. 18).

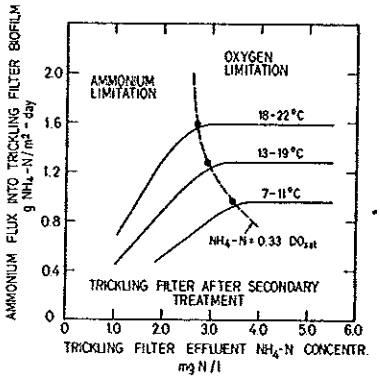



Fig. 18. Nitrification rate in trickling filters after secondary treatment. Data from USEPA-Nitrogen Control (1975).

Frank-Kamenetzkii developed a relationship that may be used to predict when the electron donor ($NH_4^+$) or the electron acceptor ($O_2$) is limiting the flux into the biofilm (1969):

$$K = \frac{D_{O_2} \cdot V_{NH_4} \cdot MW_{NH_4} \cdot S_{O_2}}{D_{NH_4} \cdot V_{O_2} \cdot MW_{O_2} \cdot S_{NH_4}}$$

where: K = dimensionless number

$D_{O_2}$, $D_{NH_4}$ = effective diffusion coefficients in the biofilm ($L^2/t$)

$V_{NH_4}$, $V_{O_2}$ = stoichiometric coefficients

$MW_{NH_4}$, $MW_{O_2}$ = molecular weights (M/mole)

$S_{O_2}$, $S_{NH_4}$ = oxygen and ammonium concentrations at the surface of the biofilm ($M/L^3$)

$K > 1$ ~~$K < 1$~~ indicates that $NH_4$ limits the nitrification rate of the biofilm, otherwise $O_2$ is limiting. Haug and McCarty (1971) theoretically predict $D_{O_2}/D_{NH_4} = 1.43$ and Wezernak and Gannon (1967) report

$$(V_{O_2} \ MW_{O_2} \ / \ V_{NH_4} \ MW_{NH_4}) = 4.33 \text{ mg } O_2/\text{mg } NH_4\text{-N}.$$

Therefore $K = 1$ if $S_{NH_4\text{-N}} = 0.33 \ S_{O_2}$

In a nitrifying trickling filter receiving secondary effluent (i.e., heterotrophic activity is low), it may be assumed that the flowing water is saturated with respect to oxygen. For this situation, the Frank-Kamenetskii relationship predicts the boundary between oxygen and ammonium limitation surprisingly well (broken line in Fig. 18).

At high ammonium concentration, the ammonium flux into the biofilm becomes independent of $NH_4$ concentration and is oxygen limited. The oxygen-limited ammonium flux is characterized by a $Q_{10} = 1.6$ which is comparable to the value of 1.86 calculated from equation (14).

This final example illustrates again the potential usefulness of fundamental data even with reference to mass transfer-limited processes.

## Conclusions

1. Much of the data in the literature on temperature effects in microbial systems reflects rates of change instead of process rates and therefore are of little value in analyzing or designing systems where physical dimensions or reaction environment are not similar.

2. Temperature effects in microbial reactions may result from both mass transfer limitations and reaction kinetics. Furthermore, sociological changes in the composition of mixed microbial populations may cause "temperature effects".

3. Substrate-limited reactions usually go to completion within the allowable retention time and are relatively unaffected by temperature.

4. All reactions that do not usually go to completion are strongly influenced by temperature, e.g., degradation of insoluble substrates, biomass decay, predation and endogenous respiration.

5. Careful analysis of a microbial system offers the possibility of estimating the effect of temperature on process performance and provides useful information even when extrapolating existing data.

6. In general, temperature does not get a great deal of attention in microbial engineering research. As a major process variable, temperature merits increased attention.

## ACKNOWLEDGMENTS

The senior author is grateful for partial support from a National Science Foundation (U.S.A.) Faculty Science Fellowship and the Swiss Federal Institute of Water Resources and Water Pollution Control. The authors acknowledge the help of Maurine Lee and Mrs. F. Schlumpf in preparing the manuscript and Miss H. Bolliger in preparing the figures.

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